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(54) PROCEDE PERMETTANT DE MODIFIER LE METABOLISME DES ACIDES GRAS CHEZ DES PLANTES
(54) METHOD TO ALTER THE FATTY ACID METABOLISM IN PLANTS

(57)

The present invention relates to amino acid and nucleic acid sequences involved in the fatty acid elongation metabolism of a plant, in particular a maize plant. Methods are disclosed to obtain plants transformed with the nucleic acid sequences of the present invention which exhibit an altered fatty acid metabolism.

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(54) PROCEDE PERMETTANT DE MODIFIER LE METABOLISME DES ACIDES GRAS CHEZ DES PLANTES

(54) METHOD TO ALTER THE FATTY ACID METABOLISM IN PLANTS

(57) The present invention relates to amino acid and nucleic acid sequences involved in the fatty acid elongation metabolism of a plant, in particular a maize plant. Methods are disclosed to obtain plants transformed with the nucleic acid sequences of the present invention which exhibit an altered fatty acid metabolism.

Abstract

The present invention relates to amino acid and nucleic acid sequences involved in the fatty acid elongation metabolism of a plant, in particular a maize plant. Methods are disclosed to obtain plants transformed with the nucleic acid sequences of the present invention which exhibit an altered fatty acid metabolism.

Method to Alter the Fatty Acid Metabolism in Plants

Description

The present invention relates to amino acid and nucleic acid sequences involved in the fatty acid elongation metabolism of a plant, in particular a maize plant, to vectors containing the nucleic acid sequences, to antibodies directed against the amino acid sequences, to methods to obtain plants transformed with the nucleic acid sequences of the present invention and to methods of use for the nucleic acid sequences of the present invention.

Seed storage lipids of higher plants are primarily made up of fatty acids containing 16 and 18 carbon atoms. These fatty acids are located in the seed oils of various plant genera. There are only a few plants, such as the Cruciferae, which accumulate oils of C20 and C22, which are referred to as very long chain fatty acids (VLCFAs). The commercial use of vegetable oils depends heavily upon the presence of VLCFAs. Erucic acid (22:1) has detrimental nutritional effects and is therefore undesirable in edible oils. Wild-type rape seed and many Brassica species, however, contain eicosenoic (20:1) and erucic acids as major components of their seed oils. However, through concerted breeding efforts, canola lines that are almost devoid of erucic acid have been developed. This has been achieved by the

introduction of recessive alleles at two loci that control the elongation of C18 fatty acids.

On the other hand, for industrial uses, vegetable oils with a high erucic acid level have been proven to be useful. These oils can be used as diesel fuel and as a raw material for an array of products, such as plastics, pharmaceuticals and lubricants. It is known that in plants de novo fatty acid synthesis (FAS) is localised in the plastids and involves intermediates bound to acyl carrier proteins (ACPs). The FAS system is believed not to produce fatty acids with chain lengths of more than 18 carbon atoms. The products of the plastid FAS are exported and converted to acyl-coenzyme A (acyl-CoA) derivatives that are thought to serve as substrates for a microsomal fatty acid elongation (FAE) system. The FAE is membrane associated and its nature and mechanism of action are partly unknown. It is believed that FAE in plants involves a four-step mechanism similar to FAS, except that CoA, rather than ACP, is the acyl carrier. Four different reactions appear to be involved in the elongation system of plants, which are (1) condensation of 18:1 CoA with malonyl-CoA to form carbon dioxide and a ß-ketoacyl-CoA in which the acyl moiety has been elongated by two carbons. Subsequent reactions are the reduction to ß-hydroxyacyl-CoA, dehydration to an enoyl-CoA and a second reduction to yield the elongated acyl-CoA. In summary, the fatty acid carbon chain is elongated from C18 to C22 by the sequential addition of two C2 moieties from malonyl coenzyme A (CoA) to a C18 carbon skeleton.

Through the development of plant genetic engineering techniques, it is possible to transform and regenerate plant species to provide plants which have novel and advantageous features. Plant genetic engineering techniques may be employed in producing insect-resistant plants, herbicide-resistant plants, draught-resistant plants and for instance also plants containing desirable products or plants devoid of undesired naturally occurring substances. Due to the potential commercial value of plants exhibiting a modified fatty acid elongation system, DNA sequences involved in the FAE system, in particular S-ketoacyl-CoA synthases (KCS) have been cloned from for instance jojoba and Arabidopsis (Lassner et al., The Plant Cell, February 1996 8, 281-292 and James et al., The Plant Cell, March 1995 7, 309-319). The seed oil of jojoba has the intrinsic feature that it consists of waxes rather than the triacylglycerols constituting other seed oils. The waxes are known to be esters of monounsaturated fatty acids and alcohols. Acyl CoA's are precursors of both the fatty acid and the fatty alcohol moieties of the wax esters. More than 90% of these fatty acids and alcohols have chain lengths longer than 18 carbon atoms, indicating the presence of an active acyl coenzyme A elongation system. As in rape seed, malonyl CoA and possibly acyl CoA serve as substrates for VLCFA synthesis. The enzyme shown to be involved in the production of the wax esters is a S-ketoacyl coenzyme A synthase involved in the acyl coenzyme A elongation pathway. Furthermore, a cDNA coding for a S-ketoacyl coenzyme A synthase from Arabidopsis was cloned and shown to be involved in the synthesis of very long

chain fatty acids in the seed (James, Jnr. et al., The Plant Cell, March 1995, 7, 309-319).

However, none of the known and cloned nucleic acid sequences involved in the fatty acyl coenzyme A elongation pathway is derived from a monocotyledonous plant. Due to the high commercial value of crop plants and differences, for instance with respect to codon usage between monocotyledonous and dicotyledonous plants, it is desirable to provide nucleic acid sequences useful for cloning genes involved in the fatty acyl coenzyme A elongation pathway in monocotyledonous plants.

The present invention solves this problem by providing a purified nucleic acid sequence for use in cloning nucleic sequences encoding a protein with the activity of a fatty acid elongase wherein the nucleic acid sequence comprises a nucleic acid sequence selected from the group consisting of

a) a nucleic acid sequence encoding any one of the amino acid sequences identified in SEQ. ID. Nos. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 35, 37 and 39 or a complementary strand or part thereof,

b) a nucleic acid sequence which hybridises to the nucleic acid sequence defined in a) or a complementary strand thereof and

c) alleles or derivatives of the nucleic acid sequences defined in a) or b).

In a particularly preferred embodiment, the present invention relates to a purified nucleic acid sequence according to the above, which is selected from the group consisting of

ai) SEQ. ID. Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 33, 34, 36 and 38 or a complementary strand or part thereof,

bi) a nucleic acid sequence which hybridises to the nucleic acid sequence defined in ai) or a complementary strand thereof,

ci) a nucleic acid sequence which is degenerated as a result of the genetic code to the nucleic acid sequence defined in ai) or bi) or a complementary strand or part thereof and

di) alleles or derivatives of the sequence defined in ai), bi) or ci).

Thus, the present invention solves the above problem by providing a DNA sequence which is in a preferred embodiment the sequence depicted in SEQ. ID. No. 32 or 33. The sequence depicted in SEQ. ID. No. 32 is a maize full length nucleic acid sequence encoding a functional protein with the activity of a fatty acid elongase. The complete amino acid sequence of this protein is depicted in SEQ. ID. No. 1. SEQ. ID. No. 33 represents the coding nucleotide sequence of the cloned nucleic acid sequence depicted in SEQ. ID. No. 32, whereby the latter additionally contains 5' and 3' non-coding sequences.

These 5' and 3' non-coding flanking sequences are also part of the present invention.

The sequences depicted in SEQ. ID. Nos. 2 to 31 and 34 to 39 are partial nucleotide and corresponding amino acid sequences. These sequences are characterised by being unique to the sequence depicted in SEQ. ID. No. 32. In fact, the sequences depicted in SEQ. ID. Nos. 2 to 31 and 34 to 39 share little, or hardly any, identity with nucleic acid sequences, or amino acid sequences being derived from plants other than maize and encoding a protein with the activity of a fatty acid elongase. Thus, these sequences are maize specific.

Thus, the present invention provides a purified nucleic acid sequence, in particular a DNA and/or RNA sequence, encoding a plant cytoplasmic protein involved in fatty acyl-CoA metabolism and/or useful for obtaining such a sequence. Of course, the sequence of the present invention is also useful for obtaining non-coding sequences, such as regulatory sequences adjacent to the sequence encoding the protein involved in fatty acyl-CoA metabolism. The nucleic acid sequences of the present invention are advantageous, as they can be used in processes to alter the composition of very long chain wax fatty acid related products such as very long chain fatty acids, in the following abbreviated as VLCFAs and/or wax esters. The nucleic acid sequences of the present invention, in particular the cDNA derived sequences, are advantageous also insofar as they allow for the production of male sterile plants and plants, for instance ornamental plants,

exhibiting a modified leaf structure, in particular modified leaf edges and a modified wax composition and/or distribution in and/or on the leaves, in particular in the cuticular.

The present invention allows for the modification of the wax composition of a transgenic plant so as to improve or create insect, fungus, pest, drought or herbicide resistance.

The present invention is particularly advantageous since the nucleic acid sequences mentioned above are specific nucleic acid sequences derived from Zea mays, that is corn or maize. Thus, the present invention provides specific maize nucleic acid sequences for use in cloning nucleic acid sequences encoding a maize protein with the activity of a fatty acid elongase. The present invention also provides nucleic acid sequences capable of enhancing, directing and/or expressing such a maize derived protein. Up to now, no nucleic acid sequences derived from a monocotyledonous plant are known capable of cloning such a nucleic acid sequence or capable of expressing such a protein.

In the context of the present invention, the term "activity of a fatty acid elongase" relates to an activity associated with the plant fatty acyl-CoA metabolism. In particular, the above activity involves condensation of malonyl-CoA with a long chain acyl-CoA to yield CO_2 and 3-hydroxyacyl-CoA, having two additional C-atoms, reduction to 3-hydroxyacyl-CoA, dehydration to enoyl-CoA and/or a further reduction to elongated acyl-CoA. Thus, the

above term relates to any one of the above activities, in particular to an activity of a protein according to which very long chain fatty acids, that is VLCFAs, are synthesised from long chain fatty acids. Thus, the protein encoded by the above DNA sequences is involved in the production of C>18, in particular, C20 and C22 and more preferred C>27, in particular C27 to C33, most preferably C28 to C30 fatty acids from fatty acids having equal or less than 18 C-atoms, in particular C16 and C18 fatty acids. In the case where fatty acids having more than 27 C-atoms are synthesised, these may be synthesised from fatty acids having more than 18 C-atoms, for instance C24 fatty acids. In particular, the protein encoded by the above nucleic acid sequences is a condensing enzyme that extends or elongates the chain length of long chain fatty acids to very long chain fatty acids. The protein encoded by the nucleic acid sequences of the present invention may, of course, also be part of an acyl-CoA elongase complex, for instance a regulatory and/or catalysing element. As explained above, the catalysing function may be a ß-ketoacyl-CoA-synthase activity. However, the protein of the present invention may also have ß-ketoacyl-CoA-reductase, ß-hydroxyacyl-CoA-dehydratase and/or enoyl-CoA-reductase activity. The protein of the present invention may also function as a fatty acyl-CoA: fatty alcohol O-acyltransforane, i.e. as a wax synthase, forming a wax ester from a fatty alcohol and a fatty acyl residue. The term "a protein with the activity of a fatty acid elongase" encompasses any one of the above described activities, either alone or in combination.

The nucleic acid sequences of the present invention may be synthetic DNA, genomic DNA or cDNA sequences. Of course, the nucleic acid sequences of the present invention also comprise RNA sequences, for instance mRNA. In addition to the sequences given in SEQ. ID. Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 33, 34, 36 or 38, the nucleic acid sequences of the present invention may contain further sequences such as regulatory elements necessary for transcription, translation, recombination or integration. These elements may already be used in addition to regulatory elements contained in the nucleic acid sequences of the present invention.

Nucleic acid sequences of the present invention include those nucleic acid sequences, that is DNA or RNA sequences, which hybridise to the specifically disclosed nucleic acid sequences of SEQ. ID. Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 33, 34, 36 or 38. Most preferably, these sequences encode a protein having the biological activity of a fatty acid elongase, in particular the maize fatty acid elongase. However, the present invention also relates to regulatory nucleic acid sequences found 5' or 3' to the coding sequences or even regulatory sequences found between the coding sequences of a genomic DNA, that means intron sequences or sequences contained in introns. The nucleic acid sequences of the present invention may, in a particular preferred embodiment, comprise sequences encoding signal or leader peptides being part of a precursor protein but not being present in the mature protein.

In the context of the present invention, nucleic acid sequences which hybridise to the specifically disclosed nucleic acid sequences of SEQ. ID. Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 33, 34, 36 or 38 are sequences which have a degree of 65% to 70% sequence identity to the specifically disclosed sequence on nucleotide level. In an even more preferred embodiment of the present invention, nucleic acid sequences which are encompassed by the present invention are sequences which have a degree of identity of more than 70%, 80% or 85% and even more preferred more than 90%, 95% or 99% to the specifically disclosed sequences on nucleotide level.

The present invention also relates to nucleic acid sequences which encode proteins, wherein the amino acid sequence of the proteins have a degree of identity of 75% to 90% on amino acid level, most preferably a degree of identity of more than 90%, 95% or 99% identity on amino acid level to the specifically disclosed amino acid sequences of the present invention.

Thus, the present invention relates to nucleic acid sequences, in particular DNA sequences which hybridise under the following conditions to the sequences specifically disclosed:

Hybridisation buffer: 1 M NaCl; 1% SDS; 10% dextran sulphate; 100 µg/ml ssDNA
Hybridisation temperature: 65° C
First wash: 2 x SSC; 0.5% SDS at room temperature

Second wash: 0.2 x SSC; 0.5% SDS at 65° C.

More preferably, the hybridisation conditions are chosen as identified above, except that a hybridisation temperature and second wash temperature of 68° C, and even more preferred, a hybridisation temperature and second wash temperature of 70° C is applied.

The present invention also comprises nucleotide rearrangements, exchanges, substitutions, insertions, deletions or modification of the above mentioned sequences as long as the biological activity of the encoded protein remains essentially the same or is improved.

As used herein, the term "promoter" refers to a sequence of DNA, usually upstream (5') to the coding sequence of a structural gene, which controls the expression of the coding region by providing the recognition for RNA polymerase and/or other factors required for transcription to start at the correct site. Promoter sequences are necessary, but not always sufficient to drive the expression of the gene. A "promoter fragment" constitutes a fraction of the DNA sequence of the promoter region.

A "3' regulatory element" (or "3' end") refers to that portion of a gene comprising a DNA segment, excluding the 5' sequence which drives the initiation of transcription and the structural portion of the gene, that contains a polyadenylation signal, also called a poly A addition sequence, and any other regulatory signals capable of affecting mes-

senger RNA (mRNA) processing or gene expression. The polyadenylation signal is usually characterised by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. Polyadenylation signals are commonly recognised by the presence of homology to the canonical form 5'-AATAA-3', although variations are not uncommon.

"Nucleic acid" refers to a large molecule which can be single or double stranded, composed of monomers (nucleotides) containing a sugar, phosphate and either a purine or pyrimidine. The term "nucleotide sequence" refers to a polymer of DNA or RNA which can be single or double stranded, optionally containing synthetic, non-natural or altered nucleotide bases capable of incorporation into DNA or RNA polymers.

As used herein, "gene" refers to a DNA sequence that codes for a specific protein and its regulatory elements.

As used herein, the term "regulatory element" refers to a nucleotide sequence located upstream (5'), within, and/or downstream (3') to a DNA sequence for a selected gene product whose transcription and/or translation is controlled by said regulatory sequence, potentially in conjunction with the protein biosynthetic apparatus of the cell. "Regulation" or "regulate" refer to the modulation of the gene expression induced by DNA sequence elements located primarily, but not exclusively, upstream of (5') the transcription start of the gene. Regulation may result in an all or none response to

a stimulation, or it may result in variations in the level of gene expression.

The term "coding sequence" refers to that portion of a gene encoding a protein, polypeptide, or a portion thereof, and excluding the regulatory sequences which drive the initiation of transcription. The coding sequence may be one normally found in the cell, in which case it is called "autologous", or it may be one not normally found in a cellular location, in which case it is termed a "heterologous gene" or "heterologous nucleic acid sequence". A heterologous gene may also be composed of autologous elements arranged in an order and/or orientation not normally found in the cell being transferred with the gene. A heterologous gene may be derived in whole or in part from any source known to the art, including a bacterial genome or episome, eukaryotic nuclear or plasmid DNA, cDNA, or chemically synthesised DNA. The structural gene may constitute an uninterrupted coding region or it may include one or more introns bounded by appropriate splice junctions. The structural gene may be a composite of segments derived from different sources, naturally occurring or synthetic.

The term "vector" refers to a recombinant DNA construct which may be a plasmid, virus, or autonomously replicating sequence, phage or nucleotide sequence, linear or circular, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along

with an appropriate 3' untranslated sequence into a plant cell.

As used herein, "plant" refers to a photosynthetic organism, such as whole plants including algae, mosses, ferns and plant-derived tissues. "Plant derived tissues" refers to differentiated and undifferentiated tissues of a plant, including but not limited to roots, shoots, leaves, pollen, ovules, tubers, tassels, seeds and various forms of cells in culture such as intact cells, protoplasts, embryos and callus tissue. Plant-derived tissues may be in planta, or in organ, tissue or cell culture. A "monocotyledonous plant" refers to a plant whose seeds have only one cotyledon, or organ of the embryo that stores and absorbs nutrients. A "dicotyledonous plant" refers to a plant whose seeds have two cotyledons.

As used herein, "transformation" refers inter alia to the processes by which cells, tissues or plants acquire properties encoded on a nucleic acid molecule that has been transferred to the cell, tissue or plant. The terms "transformation" or "transfer" refer to methods to transfer DNA into cells including, but not limited to microinjection, microprojectile bombardment, permeabilizing the cell membrane with various physical (e.g., electroporation) or chemical (e.g., polyethylene glycol, PEG) treatments.

The term "operably linked" refers to the chemical fusion of two or more fragments of DNA in a proper orientation, for instance sense or antisense orientation, such that the fusion preserves or creates a

proper reading frame, or makes possible the proper regulation of expression of the DNA sequences when transformed into plant tissue.

The term "host cell" refers to a cell which has been genetically modified by transfer of a heterologous or autologous nucleic acid sequence or its descendants still containing this sequence. These cells are also termed "transgenic cells". In the case of an autologous nucleic acid sequence being transferred, the sequence will be present in the host cell in a higher copy number than naturally occurring.

The term "expression" as used herein is intended to mean the transcription and/or translation to a gene product from a gene coding for the amino acid sequence of the gene product. In the expression, a DNA chain coding for the sequence of a gene product is first transcribed to a complimentary RNA which is often an mRNA and then the thus transcribed mRNA is translated into the above mentioned gene product if the gene product is a protein. However, expression also includes the transcription of DNA inserted in antisense orientation to its regulatory elements. Expression, which is constitutive and further enhanced by an externally controlled promoter fragment thereby producing multiple copies of mRNA and large quantities of the selected gene product, may also include over-production of a gene product. "Expression cassette" is used to refer to a DNA construct containing a promoter region operably linked to a coding sequence, which is operably linked to a 3' end and together is capable of di-

recting a mRNA of the coding region, resulting in synthesis of a mRNA and/or a protein product in plant tissue.

The term "translation start codon" or "initiation codon" refers to a unit of three nucleotides (codon) in a nucleic acid sequence that specifies the initiation of protein synthesis.

The term "signal peptide" refers to the N-terminal extension of a polypeptide, which is translated in conjunction with the polypeptide forming a precursor protein and which is required for its entrance into the secretory pathway. The signal peptide may be recognised by the mechanisms within the same species or unrelated plant species, necessary for direction of the peptide into the secretory pathway. The signal peptide may be active in seeds, leaves, tubers and other tissues of the plant. The term "signal sequence" refers to a nucleotide sequence that encodes a signal peptide. The term "vacuole targeting signal" refers to the N-terminal extension of a polypeptide, which is translated in conjunction with the polypeptide forming a precursor protein and which is required for its eventual entrance into the vacuole of a cell. The vacuole targeting signal may be recognised by the mechanisms within the same species or unrelated plant species, necessary for direction of the peptide into the vacuole of a cell. Vacuole targeting signals may be active in seeds, leaves, tubers and other tissues of the plant. The term "vacuole targeting sequence" refers to a nucleotide sequence that encodes the vacuole targeting signal.

A "tissue specific promoter" refers to a sequence of DNA which provides recognition signals for RNA polymerase and/or other factors required for transcription to start controlling expression of the coding sequence precisely within certain tissues or within certain cells of that tissue. Expression in a tissue-specific manner may be only in individual tissues or cells within tissues or in combinations of tissues. Examples may include tissue specific expression in leaves only and no other tissue within the plant, or may be in petals, ovules and stamen and no other tissues of the plant. Here, "tissue specific" is also meant to describe an expression in a particular tissue or cell according to which the expression takes place mainly, but not exclusively, in the tissue. Such an expression is also termed tissue abundant.

"Selective expression" refers to expression almost exclusively in specific organs of the plant, including leaves tubers or seeds. The term may also refer to expression at specific developmental stages in an organ, such as in early or late embryogenesis. In addition, "selective expression" may refer to expression in specific subcellular locations within the cell, such as the cytosol or vacuole.

The present invention is, of course, advantageous since the above nucleic acid sequences can be used to identify and obtain further nucleic acid sequences and consequently proteins from maize or other organisms, in particular plants, wherein the

obtained nucleic acid sequences and/or proteins are involved in fatty acyl-CoA metabolism. The nucleic acid sequences of the present invention can, of course, also be used for the construction of recombinant DNA in particular expression cassettes, for transcription and/or expression in various host organisms. The sequences of the present invention may prove particularly useful in methods to alter the VLCFA and/or wax ester composition in host cells. Preferably, the full length clone is used for purposes of expression of the protein. For the above mentioned purposes, of course, also the 5' and 3' non-coding DNA sequences of the present invention can be used for directing or enhancing the expression. The nucleic acid sequences of the present invention may be modified using standard techniques of site specific mutation or PCR. Modifications of the sequence may also be accomplished in producing a synthetic nucleic acid sequence. Such modified sequences are also encompassed by the present invention. For example, wobble positions in codons may be changed such that the nucleic acid sequence encodes the same amino acid sequence, or alternatively, codons can be altered such that conservative amino acid substitutions result. In either case, the peptide or protein maintains the desired enzymatic activity and is thus considered part of the present invention.

The present invention also relates to a vector comprising any one of the nucleic acid sequences mentioned above. Such a vector is preferably a bacterial, yeast or a viral vector, in particular a plasmid.

In a preferred embodiment of the present invention, the nucleic acid sequence of the present invention, in particular the coding sequence, in sense or antisense orientation, is operably linked to regulatory elements for directing the expression of the nucleic acid sequence, preferably in plant cells such as monocot or dicot cells or in yeast. The present invention preferably contemplates, as regulatory elements, elements that direct or enhance, in particular tissue specific, expression in the above cells. These regulatory elements may be located 5', 3' or 5' and 3' of the nucleic acid sequences, in particular the coding sequence, of the present invention. Of course, for instance in that case where a genomic DNA clone according to the present invention is used, regulatory elements may also be present within the nucleic acid sequence of the present invention, in particular within an intron. However, the regulatory element may also be an intron in its entirety.

The present invention relates in a preferred embodiment to the above mentioned vector, wherein the 5' regulatory element is a transcription initiation region, preferably a plant promoter, in particular the 35S CaMV promoter or the actin promoter of rice. However, depending upon the host and/or target tissue, the regulatory 5' element will vary and may include other regions from viral, plasmid or chromosomal genes. These genes may be derived from E. coli, B. subtilis, yeast or the like. Of course, other regulatory elements functional in plants, e.g. from plant genes, Agrobacterium tumefaciens and/or A.rhizogenes genes may also be used. The

promoters may be of inducible, regulatable, or constitutive nature. The promoter may also encompass 5' untranslated regions from foreign genes and/or translation initiation sequences.

In a further embodiment, the present invention relates to a vector, wherein the 3' regulatory element is a transcription termination region, preferably a poly A addition sequence, most preferably the poly A addition sequence of the NOS gene of Agrobacterium tumefaciens.

Of course, the present invention also relates to vectors described above, which furthermore contain further regulatory elements and/or elements necessary for the stable and/or transient integration of the nucleic acid sequence of the present invention into the genome of a host, for instance, T-DNA sequences, in particular the left, the right, or both T-DNA border sequences. In a particularly preferred embodiment of the present invention, the nucleic acid sequence of the present invention is inserted, optionally in conjunction with further regulatory elements, within the T-DNA of Agrobacterium tumefaciens or adjacent to it.

The present invention relates in a further embodiment to a host cell transformed with any one of the above mentioned vectors, in particular to a bacterial, yeast or plant cell, for instance a monocot or dicot host cell. In a particularly preferred embodiment, these host cells are capable of producing a protein with the activity of a fatty acid elongase.

The present invention also relates to cell cultures, tissues or calli comprising any one of the above host cells, in particular, these cells are capable of producing a protein with the activity of a fatty acid elongase, preferably from maize.

The present invention also relates to a protein with the activity of a fatty acid elongase produced by any one of the above mentioned host cells, in particular to a protein encoded by a nucleic acid sequence of the present invention. In the context of the present invention, the term "protein" refers to any sequence length of amino acid, irrespective of its length. Thus, within the present invention the term "protein" relates to peptides, polypetides and proteins. The protein of the present invention may be modified by addition of carbohydrates, fats or other proteins or peptides. The proteins of the present invention may also be modified by addition of isotopes, amino-, acyl-, allyl-, or other groups.

A particularly preferred amino acid sequence of the present invention is any one of the amino acid sequences of SEQ. ID. Nos. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 35, 37 or 39.

The present invention also relates to an antibody, in particular a monoclonal or polyclonal antibody, which is reactive with the protein of the present invention. These antibodies may be used to screen expression libraries to identify clones which produce similar or related plant cytoplasmic proteins involved in fatty acyl-CoA metabolism.

The present invention also relates to a method of genetically modifying a cell, preferably a plant, bacterial or yeast cell, by transforming the cell with a vector of the present invention, whereby the nucleic acid sequence contained in the vector is expressible in the cell. Thus, the present invention also relates to a method of transformation of a cell, in particular a plant, bacterial or yeast cell. The cells to be transformed may be cells which do not possess an endogenous DNA sequence encoding a protein with the activity of a fatty acid elongase, in particular of a maize fatty acid elongase. However, the present invention also relates to the transformation of cells which do possess an endogenous DNA sequence encoding a protein with the activity of a fatty acid elongase, in particular a maize fatty acid elongase. In this embodiment, the DNA sequence of the present invention is particularly preferred under the control of regulatory elements not associated originally in the cells to be transformed with the DNA sequence of the present invention and/or the DNA sequence of the present invention is preferably used as an antisense construct. Of course, the transformation process of the present invention is not limited only to cells themselves, but may also be applied to tissues, plant parts such as cotyledons or petioles or calluses or embryos.

The present invention also relates to plants and processes to obtain plants, wherein a DNA construct comprising a nucleic acid sequence of the present invention cloned in sense or antisense orientation under the control of appropriate regulatory ele-

ments is transformed in a plant cell so as to eliminate or reduce the wild-type expression of an autologous or endogenous gene encoding a fatty acid elongase. Such an eliminating effect may be obtained via antisense constructs or sense constructs, wherein the latter lead to cosuppression, for instance due to a high copy number. In particular, expression of the transgenic copy or copies, i.e. the transformed nucleic acid sequence, may not be necessary and the mere presence of the transformed nucleic acid sequence(s) may be sufficient to cause alteration of the very long chain fatty acids and/or waxes amount or composition.

The transformation may be carried out with plant species which are naturally susceptible to Agrobacterium tumefaciens or Agrobacterium rhizogenes infection via methods of Agrobacterium mediated transformation. Of course, other transformation methods can also be used, such as direct uptake of DNA by microinjection or particle bombardment. Of course, any further methods can also be used, such as electroporation methods or the use of plant pathogenic viruses or plant transposable elements. After transformation, the preferred plant cells of the present invention are cultivated and regenerated to intact, fertile plants via conventional methods.

The present invention of course also relates to plants comprising genetically modified cells according to the present invention, capable of expressing and/or possessing the incorporated nucleic acids of the present invention, in particular

seeds, embryos, calluses, cotyledons, petioles and plant tissue, harvest material and reproductive tissue derived from such a plant or used to produce a plant and still comprising at least one genetically modified cell.

Thus, the present invention relates to seeds, plant parts and embryos, non-biologically transformed, which possess, stably integrated in the genome of their cells, a preferably heterologous, nucleic acid sequence of the present invention containing a promoter recognised by the polymerases of the cells of said seeds, plant parts or embryos and the nucleic acid sequence of the present invention encoding a protein having a non-variety-specific enzymatic fatty acid elongase activity or part thereof or being an antisense construct.

Thus, the invention also relates to plants or plant parts or plant tissue, calluses, embryos, cotyledons or petioles, non-biologically transformed, which possess, stably integrated in the genome of their cells, a, preferably heterologous, nucleic acid sequence encoding a protein having a non-variety-specific enzymatic fatty acid elongase activity or part thereof or being an antisense construct under the control of a promoter recognised by the polymerases of said cells.

In the case where the nucleic acid sequence of the present invention is not heterologous but autologous to the transformed host, the regulatory elements associated with the transformed DNA sequence of the present invention and/or the orientation of

the DNA sequence of the present invention in relation to its promoter and/or its integration location in the genome and/or the copy number in the transformed cell are most preferably different from the untransformed host.

The teaching of the present invention is therefore applicable to any plant, plant genus or plant species, wherein the fatty acyl-CoA metabolism is to be modified, for instance maize, rice, wheat, barley, rape, peanut, oat, rye, pea, soybean, potato, sugar beet, sugar cane, sorghum, Brassica, tobacco, sunflower, carrot, tomato, cucumber, cotton, poplar, dactylis, Festuca, Lolium, Arabidopsis, lettuce, ornamental plants, etc.

Finally, the present invention relates to a method of modifying the content of very long chain fatty acid molecules, in particular with C>27, in a plant cell, wherein a plant cell is transformed with a nucleic acid sequence of the present invention and the plant cell, in particular a plant regenerated from the plant cell, is grown under conditions wherein the transformed nucleic acid sequence is expressed or wherein the transformed nucleic acid sequence is present and interferes with endogenous expression of an endogenous gene encoding a fatty acid elongase.

The present invention, of course, also relates to the production of VLCFAs, in particular with C>27, or modifications of its amounts of such fatty acids in host cells. Thus, the present invention provides for such a method wherein an increased production

of VLCFAs, in particular with C>27, in the host cell may be obtained by expression of nucleic acid sequences of the present invention. The present invention of course also relates to such a method wherein antisense constructs or sense constructs used in cosuppression technology containing sequences of the present invention are used to reduce and/or alter the content of VLCFAs, in particular with C>27, in a host cell. Thus, the present invention advantageously provides new plants and plant seeds and in particular plant seed oils with a desirable fatty acid composition.

The present invention also relates to a method for producing VLCFA's, in particular with C>27, and/or waxes in a plant comprising producing a genetically modified plant of the present invention, harvesting the plant and extracting the VLCFA's and/or waxes from the harvested plant.

The present invention also relates to the production of wax esters or modifications of its amounts in host cells of the present invention. The host cells used for the production or modification of the amount of wax esters should contain fatty acyl and fatty alcohol substrates for the wax synthase activity of the present invention.

Further preferred embodiments of the present invention are specified in the appended claims.

The present invention is explained in more detail by the sequence listing which is part of the present description.

SEQ. ID. No. 1 depicts the deduced full length amino acid sequence coded by ZmKCS1 having 494 amino acids.

SEQ. ID. Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 34, 36 and 38 depict partial nucleotide and deduced amino acid sequences coded by ZmKCS1. In detail and in reference to the nucleotide numbering of the full length nucleotide sequence of ZmKCS1 depicted in SEQ. ID. No. 32. SEQ. ID. No. 2 represents the nucleotides from and including position 138 to 161, SEQ. ID. No. 4 from position 171 to 215, SEQ. ID. No. 6 from position 225 to 296, SEQ. ID. No.8 from position 306 to 383, SEQ. ID. No. 10 from position 429 to 512, SEQ. ID. No. 12 from position 540 to 632, SEQ. ID. No. 14 from position 885 to 935, SEQ. ID. No. 16 from position 981 to 1013, SEQ. ID. No. 18 from position 1077 to 1109, SEQ. ID. No. 20 from position 1119 to 1154, SEQ. ID. No. 22 from position 1212 to 1256, SEQ. ID. No. 24 from position 1458 to 1490, SEQ. ID. No. 26 from 1524 to 1571, SEQ. ID. No. 28 from position 1593 to 1616, SEQ. ID. No. 30 from position 1566 to 1580, SEQ. ID. No. 34 from position 633 to 665, SEQ. ID. No. 36 from position 1053 to 1070 and SEQ. ID. No. 38 from position from position 1344 to 1358.

SEQ. ID. Nos. 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 35, 37 and 39 depict partial amino acid sequences of ZmKCS1 deduced from the above partial nucleotide sequences.

SEQ. ID. No. 32 depicts the full length nucleotide sequence from position 1 to position 1954 of clone ZmKCS1 comprising a nucleotide sequence encoding the amino acid sequence of SEQ. ID. No. 1.

SEQ. ID. No. 33 exclusively depicts the coding region of the nucleotide sequence of SEQ. ID. No. 32.

The present invention will now be more specifically described in the following examples and the accompanying drawings.

Figure 1 presents a restriction map of the DNA construct pAct1.D16.3α.

Figure 2 presents a restriction map of the DNA construct pBHT9.1.

Example 1:

Isolation of the *ZmKCS1* cDNA clone:

A cDNA bank was constructed from RNA isolated from maize kernels of the line *cl-p* (Chen and Coe, 1977). Messenger RNA was isolated from the kernels that were germinated under light for 0, 3 and 6 hours. cDNA from this mRNA was generated with the Stratagene ZAP-cDNA-Synthesis kit following manufacturer's protocols. Upon size fractionation, the cDNA fragments longer than 500 bp were ligated into the vector λUni-ZAP XR (Stratagene) as suggested by the manufacturer. The resulting DNA molecules were packaged into phage particles using the Gigapack II-Gold Packaging Extracts as suggested by the

manufacturer. The titer of the unamplified bank was 1,000,000 recombinant plaque forming units (pfu). This bank was amplified before screening.

The differential screening of a total of 60,000 pfu of the cDNA mentioned above was performed as follows: twenty four plates (150 mm in diameter) were prepared with 2,500 pfu per plate. These plates were lifted twice with nitrocellulose filters. These filter-replicates were hybridised under stringent conditions, i.e. hybridisation buffer: 1 M NaCl, 1% SDS, 10% dextran sulphate, 100 µg/ml single stranded salmon sperm DNA; hybridisation temperature 65° C, first wash 2 x SSC; 0.5% SDS at room temperature; second wash: 0.2 x SSC; 0.5% SDS at 65° C, with a radioactively labelled single-stranded cDNA preparation from mRNA isolated from maize kernels germinated either in the dark or in the presence of light for 3 and 6 hours, respectively.

One clone was isolated from this differential screening, *D16.3*, that carried an insert with high sequence identity to known KCS (James et al., The Plant Cell, March 1995 7, 309-319 and Lassner et al., The Plant Cell, February 1996 8, 281-292) genes at the nucleotide and deduced amino acid levels. Since this clone did not contain the putative initiation codon of the longest open reading frame, 1,200,000 pfu of the same cDNA bank mentioned above were hybridised under the above described stringent conditions with the insert of the clone *D16.3* in order to isolate a clone containing the complete coding region. One isolated clone, *9.1*, carried an

insert whose nucleotide sequence encompassed, with complete identity, the complete sequence of the insert of *D16.3* as well as the full coding region. This clone was renamed *ZmKCS1*. The full length nucleotide sequence of clone ZmKCS1 is depicted in SEQ. ID. No. 32. The sequence contains a 5' non-coding region from positions 1 to 134. A translation initiation codon ATG is located at position 135 to 137. The translation stop codon TGA is located at position 1617 to 1619 whilst from position 1620 to position 1954 a 3' untranslated non-coding region is located. SEQ. ID. No. 33 represents those nucleotides, i.e. nucleotides 135 to 1616, which encode the amino acid sequence depicted in SEQ. ID. No. 1. SEQ. ID. Nos. 2 to 31 and 34 to 39 represent partial DNA and amino acid sequences being derived by sequence comparisons with genes which are thought to encode proteins with similar activity from Arabidopsis, jojoba, Brassica napus and Brassica juncea. The partial nucleotide and amino acid sequences SEQ. ID. Nos. 2 to 31 and 34 to 39 of the present invention share hardly any, or no, sequence identity with corresponding known sequences from other species and are therefore particularly suitable for cloning purposes in monocotyledonous plants, in particular in maize.

The cDNA clone ZmKCS1 has been used in northern experiments with maize tissue and shows expression in germinating kernels, predominantly in endosperm tissue, and young seedlings as compared to adult leaves with almost no expression. In tassel tissue strong expression could also be observed.

Example 2:

Modification of the VLCFA composition of plants: Generation of transgenic plants carrying a *ZmKCS1* antisense or sense construct:

2.1 Transformation of monocot plants (e.g. maize):

The VLCFA and/or wax composition of a maize plant may be altered by the antisense technology and clone D16.3. Upon inhibition of the expression of the ZmKCS1 gene, the length of the carbon chains of the above mentioned compounds will be significantly reduced.

The 1.2-kb insert of the clone pD16.3 was cloned into a unique cloning site of the expression vector pActl.cas (Cambia TG 0063) (this vector contains the promoter and first intron of the actin1 gene of rice; McElroy et al., 1990) in antisense orientation in relation to the promoter. The resulting 5.2 kb construct, pAct1.D16.3α (figure 1), was used to transform, via a particle bombardment protocol (Brettschneider, Becker and Lörz 1997), scutelar tissue of immature embryos of the hybrid maize line A188xH99.

1.0-1.4 mm long immature maize embryos were isolated and cultured on nutrient medium. The embryos were bombarded 4 to 10 days after isolation. The bombardment was performed using a PDS 1000/He gun (BioRad). The gold particles, 0.4-1.2 µm in diameter, were accelerated onto the plant material using rupture disks of 1350 psi. For selection of the

transformed tissue, a construct (p35S-PAT; P.Eckes), harbouring a selectable marker conferring resistance to the drug phosphinotricin (PPT), was co-transformed with pAct1.D16.3α.

Plants were regenerated on culture media supplemented with 1 mg/ml PPT and were transferred to soil after reaching 10 cm in height.

Southern blot analysis with genomic DNA isolated from transformed regenerated maize plants showed the presence of the transformed construct.

The screening of the transformants involved the determination, via Northern blots, of the effectiveness of the antisense method. A reduction of the amount of ZmKCS1 mRNA could be observed. Furthermore, the VLCFA/wax composition was analysed by gas-chromatography. It could be shown that the lengths of the carbon chain of VLCFAs and waxes present in the transgenic maize plants were drastically reduced. Also, the shape of leaves was drastically changed and most plants were male sterile. The transformed maize plants were effected primarily in the development of the leaf structure. Leaf development showed a characteristic misformation of the outer areas of leaves. Some of the transformed plants were very small in growth and most plants were negatively effected in tassel and ear development. Also, most plants did not develop intact anthers and pollen development was strongly reduced or absent. Pollen shedding was also effected and microscopical analysis of pollen grains revealed that many pollen grains were aborted.

Southern analysis of maize and tobacco plants revealed that the plants were transformed and contained sequences of the construct used.

2.2 Transformation of dicot plants (e.g. tobacco):

Analogously to the experimental set-up of example 2.1, the VLCFA/wax composition of a tobacco plant was altered. The insert of the clone ZmKCS1 was cloned in a binary vector in sense orientation in relation to the 35S promoter of CaMV. The resulting 14.9 kb construct pBHT9.1 (figure 2) was transformed into *Agrobacterium tumefaciens* and used to transform leaf-discs of tobacco. Transformation and regeneration of transformed plants was carried out following established protocols.

Southern blot analysis with genomic DNA isolated from transformed and regenerated tobacco plants showed the presence of the transformed construct.

The analysis of the transformants has been done as mentioned above. The tobacco plants obtained showed a severe reduction in the amount of ZmKCS1 mRNA and a reduction in the lengths of carbon chains in the VLCFAs and waxes present in the tobacco plants obtained. The results show that possibly a cosuppression effect can be obtained using a sense orientation of the nucleic acid sequence of the present invention.

Example 3:

Isolation of new genes involved in fatty acid elongation in maize through a direct/interaction approach:

ZmKCS1 is a member of a gene family in maize. cDNA clones which are similar in the coding region but vary widely in the 3' untranslated region, have been isolated according to the present invention, signifying that they derived from different genes. The working hypothesis is that this large gene family includes condensing enzymes participating in specific elongation steps and in different tissues, e.g. seed and leaf. The fact that ZmKCS1 is also expressed in leaves, allows direct probing of the condensing step of epicuticular wax biosynthesis. The genes for all other condensing enzymes characterised to date are solely expressed in seeds.

It is preferred to use an oligonucleotide comprising the sequence 5'-GACGACTGCATCCAC-3' (SEQ ID. No. 30) to screen, via PCR, various cDNA banks constructed with mRNA from various tissues, especially from leaves of different ages, in order to find the active condensing enzymes in those tissues.

The fact that the four enzyme activities catalysing fatty acid elongation interact physically in a tightly-bound complex may be used to isolate clones coding the other three enzymatic activities. It is preferred to use the "Two Hybrid Screening" (Stratagene) using ZmKCS1 and the other identified condensing enzymes as baits to isolate their inter-

acting partners. The screening would be repeated until all four partners had been identified.

Example 4:

Functional analysis of the ZmKCS1 protein:
expression of ZmKCS1 in yeast
(Saccharomyces cerevisiae)

To determine the enzymatic activity of the protein ZmKCS1, a yeast strain (INVSc-1; Invitrogen) was transformed with a construct expressing ZmKCS1. The influence that the presence of this protein in yeast cell has on the fatty acid composition was assessed.

These experiments are carried out as follows by use of PCR technology, the start codon of ZmKCS1 has been modified to fit the yeast consensus (Cigan and Donahue, 1987) and a restriction site for cloning has been incorporated immediately 3' of the stop codon. The resulting fragment was cloned in pBS+ (Stratagene) to generate the construct pB9.1M. The correctness of its sequence was determined upon sequencing. The fragment from pB9.1M was cloned in the expression vector PYes2 (Invitrogen) and the resulting construct pY9.1M was then transformed in the yeast strain.

In pY9.1M the expression of the ZmKCS1 gene is under the control of a strong, galactose-inducible promoter. The transformed yeast cells were grown on a galactose-free medium and the expression of ZmKCS1 was induced upon addition of galactose. Af-

ter further culturing, the cells were harvested and its lipids extracted and analysed through gas-chromatography.

Example 5:

Over-expression and production of polyclonal antibodies against the protein ZmKCS1:

In order to isolate sufficient quantities of the protein ZmKCS1 to raise polyclonal antibodies, the nucleotide sequences coding for this protein was cloned in an expression vector of the series pRSET (Invitrogen). These vectors allow over-expression of proteins in *E. coli* under the control of the bacteriophage T7 promoter. The nucleotide sequence of ZmKCS1 was modified by PCR to allow the cloning, in frame, of the open reading frame coding for ZmKCS1 with the bacterial start codon present in the vector.

The resulting construct was transformed into an *E. coli strain* that carries the gene encoding the bacteriophage T7 RNA polymerase under the control of the IPTG-inducible *lac* promoter. Addition of IPTG to the culture medium results in a strong and rapid induction of the expression of the ZmKCS1. The resulting protein also carries an amino acid tag consisting of six histidines. This allows the ready isolation of the tagged proteins by chromatography on niquel columns.

The purified ZmKCS1 protein may be used to raise antibodies in, for example, rabbits.

These antibodies may be helpful in, for example, the identification of clones which express ZmKCS1-similar proteins and the determination of this protein in planta.

References:

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Chen, S. and Coe, E.H. 1977. Control of anthocyanin synthesis by the C-locus in maize. Biochem. Genetics 15: 333-346

Cigan, A.M. Donahue, T.F. 1987. Sequence and structural features associated with translational initiator regions in yeast - a review. Gene 59: 1-18.

McElroy, D., Zhang, W., Cao, J. and Wu, R. 1990. Isolation of an efficient actin promoter for use in rice transformation. Plant Cell 2: 163-171.

SEQUENCE LISTING

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Claims

1. A nucleic acid molecule for use in cloning nucleic acid molecules encoding a protein with the activity of a fatty acid elongase wherein the nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of

a) the nucleic acid sequence encoding any one of the amino acid sequences identified in SEQ. ID. Nos. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 35, 37 and 39 or a complementary strand thereof,

b) a nucleic acid sequence which has a degree of identity of more than 70% to the nucleic acid sequence defined in a) and

c) alleles of the nucleic acid sequence defined in a) or b).

2. The nucleic acid molecule according to claim 1, which is selected from the group consisting of

a) SEQ. ID. Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 33, 34, 36 and 38 or a complementary strand thereof,

b) a nucleic acid sequence which has a degree of identity of more than 70% to the nucleic acid sequence defined in a),

c) a nucleic acid sequence, which is degenerate as a result of the genetic code to the nucleic acid sequence defined in a) or b) and

d) alleles of the sequence defined in a), b) or c).

3. The nucleic acid sequence according to any one of the preceding claims, which is a Zea mays nucleic acid sequence.

4. A vector comprising the nucleic acid sequence of any one of the preceding claims.

5. The vector of claim 4, which is a bacterial, yeast or a viral vector.

6. The vector according to claim 4 or 5, which is a plasmid.

7. The vector according to claim 5 or 6, wherein the nucleic acid sequence of any one of claims 1 to 3 is operably linked to regulatory elements for directing the expression of the nucleic acid sequence in a monocot or dicot plant cell or a yeast.

8. The vector of claim 7, wherein the regulatory elements direct or enhance tissue specific expression in plants.

9. The vector of claim 8, wherein the regulatory elements direct or enhance tissue specific expression in monocot plants.

10. The vector of any one of claims 4 to 9, wherein the regulatory elements are 5', 3' or 5' and 3' regulatory elements.

11. The vector of claim 10, wherein the 5' regulatory element is a plant promoter.

12. The vector of claim 11, wherein the 5' regulatory element is the 35S CaMV promoter.

13. The vector of any one of claims 10 to 12, wherein the 3' regulatory element is a transcription termination region.

14. The vector of claim 13, wherein the 3' regulatory element is a poly A addition sequence.

15. The vector of claim 14, wherein the 3' regulatory element is the poly A addition sequence of the NOS gene of Agrobacterium tumefaciens.

16. The vector of any one of claims 4 to 15, wherein the nucleic acid sequence is operably linked to a targeting sequence.

17. The vector according to any one of claims 4 to 16, which furthermore contains the left, the right or both T-DNA borders of T-DNA.

18. The vector according to any one of claims 4 to 17, wherein the nucleic acid sequence is inserted within the T-DNA or adjacent to it.

19. The vector of claim 18, wherein the nucleic acid sequence is inserted in conjunction with the regulatory elements within the T-DNA or adjacent to it.

20. A host cell transformed with the vector of any one of claims 4 to 19.

21. The host cell of claim 20, which is a plant, yeast or bacterial cell.

22. The host cell of claim 21, which is a monocot or dicot cell.

23. A cell culture, comprising a cell according to any one of claims 20 or 22.

24. A cell culture according to claim 23, which is a plant, yeast or bacterial cell culture.

25. A cell culture comprising plant, yeast or bacterial cells, which are capable of producing a protein with the activity of a fatty acid elongase.

26. A cell culture according to claim 25, comprising plant, yeast or bacterial cells, which are capable of producing a protein with the activity of a fatty acid elongase from Zea mays.

27. A protein with the activity of a fatty acid elongase produced by any one of the cells of claims 20 to 22 from Zea mays.

28. A protein having the activity of a fatty acid elongase and being encoded by any one of the sequences given in claims 1 to 3.

29. An antibody, which is reactive with the protein of claim 27 or 28.

30. A plant comprising a genetically modified cell according to any one of claims 20 to 22.

31. A plant comprising a genetically modified cell according to any one of claims 20 to 22, which is Zea mays.

32. Seeds and plant tissue comprising a genetically modified cell according to any one of claims 20 to 22, from a plant according to claim 30 or 31.

33. A method of genetically modifying a cell by transforming the cell with a vector according to any one of claims 4 to 19, whereby the nucleic acid sequence contained in the vector is expressible in the cell.

34. The method of claim 33, wherein the cell is a plant, bacterial or yeast cell.

35. The method of claim 34, wherein the transformed cell is regenerated to a differentiated plant.

36. The method of claim 34, wherein the cell is transformed by transfer of nucleic acid sequences from a bacterium to the cell.

37. The method of claim 34, wherein the cell is transformed by direct uptake of nucleic acid sequences, by microinjection of nucleic acid sequences or by particle bombardment.

38. A method of modifying the content of very long chain fatty acid molecules and/or waxes in a plant cell, wherein a plant according to claim 30 or 31 or a plant being transformed according to any one of claims 33 to 37 is grown under conditions, wherein the transformed nucleic acid sequence is present or expressed.

39. The method of claim 38, wherein the proportion of very long chain fatty acid molecules and/or waxes is changed and wherein the nucleic acid sequence expresses a protein such as to produce from long chain fatty acid acyl-CoA molecules very long chain fatty acid molecules and/or waxes.

40. The method of claim 39, wherein the proportion of very long chain fatty acid molecules and/or waxes is increased.

41. The method of claim 38, wherein the proportion of very long chain fatty acid molecules and/or waxes from a given proportion is decreased and wherein the nucleic acid sequence is cloned in antisense or sense in the vector and represses the

production of a protein involved in producing very long chain fatty acid molecules and/or waxes.

42. A method of producing very long chain fatty acid molecules and/or waxes in a plant, comprising growing the plant of claim 20 or 31, harvesting the plant and extracting the very long chain fatty acid molecules and/or waxes from the harvested plant.

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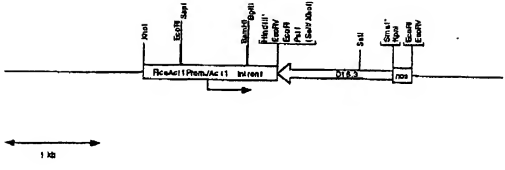

Figure 1

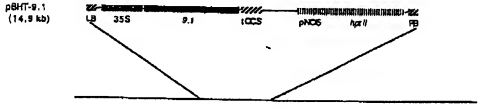

Figure 2

Marks & Clerk